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Emergence and phytopathological properties of a new strain of *Claviceps purpurea* (Fr.) Tul. on rye

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SUMMARY

The emergence of a new stable strain of *Claviceps purpurea* is described. The strain formed the sphacelial stage very weakly on rye, and was unlikely therefore to be able to initiate an epidemic under field conditions, but the sclerotia contained chanoclavine as the major alkaloid in contrast to the ergotamine component of the parent strain. Isolates of *C. purpurea* derived from ryegrass ergots were also shown to parasitize rye.

INTRODUCTION

It is generally thought that the qualitative alkaloidal spectrum of ergot sclerotia is a character of the fungal strain rather than of the host plant on which the fungus may be parasitic. Thus, although there may be a quantitative variation in the total alkaloid content on various host grasses, the constituent alkaloids are usually qualitatively the same.

This paper describes the emergence of a stable strain of *C. purpurea* which produced sclerotia on rye in which the alkaloid components were both qualitatively and quantitatively quite different from the parent strain.

MATERIALS AND METHODS

The original sample of ryegrass (*Lolium perenne* L.) ergots (sample 84) upon which this study was based was collected from a permanent pasture in South Wales in January 1964.

The isolates of *C. purpurea* were obtained from sclerotial fragments on potato dextrose agar after surface sterilizing with mercuric chloride (0·1 %) for 5 min. Isolates were transferred to and maintained on agar slopes of a standard asparagine/sucrose medium (Castagnoli & Mantle, 1966). Aqueous conidial suspensions, washed from the surface of agar cultures, were injected by hypodermic syringe into rye florets before anthesis. In the greenhouse, ears were covered with polythene sleeves for 7 days after inoculation to prevent cross-infection. Small garden plots of rye were located well away from any indigenous ergot and ears were inoculated in early June before any natural sphacelial epidemics could have developed on wild grasses. Sclerotia were harvested when the ears were ripe.

The alkaloid content of sclerotia was determined by extracting, five times with ether, a finely ground aliquot which had been mixed to a wet paste with sodium bicarbonate.

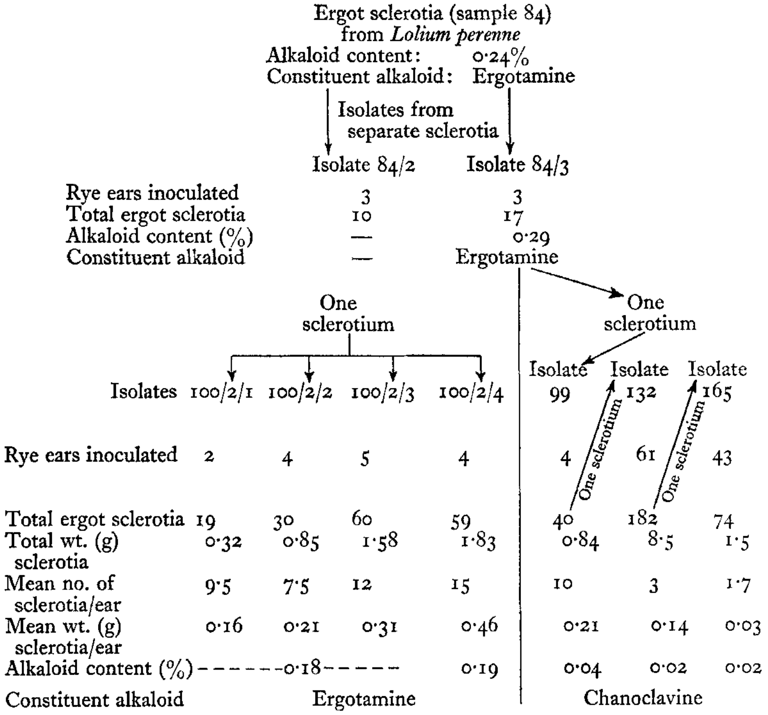
The combined ether extract was shaken out with 1 % tartaric acid, and this was assayed colorimetrically by comparing, with a standard alkaloid solution, the blue colour developed after mixing one volume of alkaloid extract with two volumes of van Urk reagent (0·125 % *p*-dimethylaminobenzaldehyde in 65 % $H_2SO_4+0{\cdot}1$ % of 5 % aqueous $FeCl_3$).

Determination of the alkaloidal constituents was initially by descending paper chromatography (Foster, Macdonald & Jones, 1949; Pohm & Fuchs, 1954), and later by thin-layer chromatography on Silica Gel G with ethyl acetate/ethanol/dimethylformamide 13:0·1:1·9 (McLaughlin, Goyan & Paul, 1964) and chloroform/methanol 4:1 (Agurell, Ramstad & Ullstrup, 1963) as solvent systems.

EXPERIMENTAL

The sequence by which the new strain was obtained is summarized in Table 1.

Table 1. *Sequence of development on rye of a new strain of* C. purpurea (*containing chanoclavine as the major alkaloid*) *derived from a sample of ryegrass ergots* (*containing ergotamine as the major alkaloid*)



Ergot sclerotia (sample 84)
from *Lolium perenne*
Alkaloid content: 0·24%
Constituent alkaloid: Ergotamine

	Isolate 84/2	Isolate 84/3
Rye ears inoculated	3	3
Total ergot sclerotia	10	17
Alkaloid content (%)	—	0·29
Constituent alkaloid	—	Ergotamine

Isolates	100/2/1	100/2/2	100/2/3	100/2/4	Isolate 99	Isolate 132	Isolate 165
Rye ears inoculated	2	4	5	4	4	61	43
Total ergot sclerotia	19	30	60	59	40	182	74
Total wt. (g) sclerotia	0·32	0·85	1·58	1·83	0·84	8·5	1·5
Mean no. of sclerotia/ear	9·5	7·5	12	15	10	3	1·7
Mean wt. (g) sclerotia/ear	0·16	0·21	0·31	0·46	0·21	0·14	0·03
Alkaloid content (%)	0·18			0·19	0·04	0·02	0·02
Constituent alkaloid	Ergotamine				Chanoclavine		

The isolates 84/2 and 84/3 apparently differed in their ability to produce typical sclerotia in rye inflorescences. It was not clear whether this was a reflection of their infectivity or of their subsequent ability to become well-established parasites and to form sclerotia. Isolate 84/2 produced fewer sclerotia than isolate 84/3 and they were distinctly smaller, but on isolating from four separate parts of one of the larger sclerotia (isolates 100/2/1–4) all reisolates subsequently produced sclerotia more readily on rye than the parent. The alkaloid content of these sclerotia was only slightly less than that of the original sample and had the same major component—ergotamine. Traces of the isomer ergotaminine were observed on chromatograms but this was possibly a product of analysis, and therefore an artifact. It was concluded that the derivatives of isolate 84/2 were typical of *C. purpurea* on the rye host both with regard to the formation of sclerotia and to their alkaloid content.

Isolate 84/3 was the ryegrass isolate which showed the greatest ability to form sclerotia on rye. The quantity of sclerotia produced was sufficient for determination of the alkaloid content, which was found to be similar both in amount and type to the original ryegrass sample. However, a re-isolate from one of these sclerotia (isolate 99) produced sclerotia similarly on the rye host but the alkaloid content was about one-sixth of normal and consisted almost entirely of chanoclavine. No trace of ergotamine was detected. Re-isolation from a sclerotium of this new type gave a strain (isolate 132) which, though inoculated rather less precisely than in previous tests into only a few florets on each of sixty-one rye ears in a small garden plot, gave good sclerotia though with an alkaloid content only about 10% of normal and again consisting of chanoclavine. By isolating from one of these sclerotia (isolate 165), and repeating the procedure in the following year, similar sclerotia were obtained with a similar alkaloid component. It was particularly noticed, however, that following inoculation of rye with isolates 132 and 165 there was no normal exudate of honeydew from those florets from which later the sclerotia gradually emerged. However, the presence of some conidia on the surface of the sclerotia at maturity indicated that some sphacelial fructification had occurred. Five months after harvest, sclerotia of isolate 165 were treated for 4 weeks at 3 °C on moist sand and subsequently germinated after a further 2 months at room temperature.

Parallel with this series of experiments all new isolates were tested for alkaloid production in surface culture on the asparagine/sucrose broth but all strains produced thin surface mats on which extensive sporulation occurred, and no traces of alkaloid were detected in the broth.

DISCUSSION

The means by which this new strain arose is not clear but, in that chanoclavine is thought to be an intermediate in the biosynthesis of lysergic acid derivatives (Floss *et al.* 1967), it may represent a variant having a metabolic block resulting in synthesis of the ergoline nucleus only as far as chanoclavine.

It is generally thought that British strains of *C. purpurea* parasitizing ryegrass are unable also to parasitize rye (Moore & Moore, 1964). In the present experiment, although there was some variation in the degree of parasitism of the three isolates from separate sclerotia of the original ryegrass sample, it was clear that parasitism under the conditions of artificial conidial inoculation did occur quite readily. Other ryegrass isolates have also been found to parasitize rye (Mantle, unpublished). Agriculturally, therefore, ryegrass infected with *C. purpurea* must now be regarded as a potential danger to rye. However, a number of other factors may be limiting, such as the coincidence of rye flowering with available inoculum on ryegrass, and environmental conditions at anthesis. The original sample of ergotized ryegrass had earlier (September 1963) been associated with a severe case of gangrenous ergotism in cattle, which was investigated and subsequently described by Woods, Bradley-Jones & Mantle (1966). Although a direct causal relationship between the ergot and the clinical condition of the cattle could not be absolutely proven, very strong circumstantial evidence of a connexion was presented. Thus the present study can most probably be directly related to a specific outbreak of gangrenous ergotism and, particularly with regard to the pathogenicity of the ryegrass isolates on rye, is an extension of the phytopathological and epidemiological aspects of this case of ergot poisoning.

The original ryegrass isolates were clearly able also to form the characteristic alkaloid component while parasitizing the rye floret, but subsequently a stable strain arose which produced an abnormally low yield of alkaloid consisting not of a lysergic acid derivative but of a clavine alkaloid. It is significant that although this new strain sporulated very freely *in vitro*, it produced only a trace of the sphacelial stage on the rye host, and this was observed only as spores on the sclerotial surface and not in the usual heavy spore load of the honeydew exudate. Although this strain lacked the ability for secondary conidial spread on rye, the sclerotia remained viable until the following year and were potentially able to initiate primary ascospore infections. Thus it is probable that if this strain had arisen under field conditions it would not have spread sufficiently to become significantly established, as it lacked the means for producing an epidemic in competition with normal strains. This characteristic may explain why this is, as far as is known, the first record of chanoclavine as the major alkaloid component of sclerotia of *C. purpurea.*

REFERENCES

AGURELL, S. L., RAMSTAD, E. & ULLSTRUP, A. J. (1963). The alkaloids of maize ergot. *Planta med.* 4, 392–8.

CASTAGNOLI, N. & MANTLE, P. G. (1966). Occurrence of D-lysergic acid and 6-methyl-ergol-8-ene-8-carboxylic acid in cultures of *Claviceps purpurea. Nature, Lond.* **211**, 859–60.

FLOSS, H. G., HORNEMAN, U., SCHILLING, N., GROGER, D. & ERGE, D. (1967). Chanoclavines and the biosynthesis of ergot alkaloids. *Chem. Commun.* 3, 105–6.

FOSTER, G. E., MACDONALD, J. & JONES, T. S. G. (1949). The separation and identification of ergot alkaloids by paper partition chromatography. *J. Pharm. Pharmac.* 1, 802–12.

MCLAUGHLIN, J. L., GOYAN, J. E. & PAUL, A. G. (1964). Thin-layer chromatography of ergot alkaloids. *J. Am. pharm. Ass.* 53, 306–10.

MOORE, W. C. & MOORE, F. J. (1964). Cereal diseases. *Bull. Minist. Agric. Fish. Fd.* no. 129, 43 pp.

POHM, M. & FUCHS, L. (1954). On the paper chromatographic separation and estimation of ergot alkaloids. *Naturwissenschaften* 41, 63.

WOODS, A. J., BRADLEY-JONES, J. & MANTLE, P. G. (1966). An outbreak of gangrenous ergotism in cattle. *Vet. Rec.* 78, 742–9.